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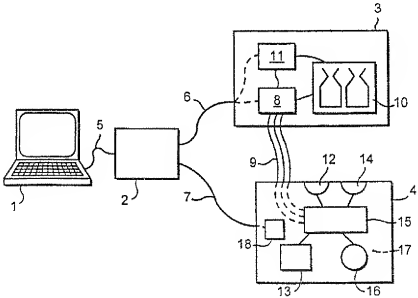

(57) Abstract

Apparatus for performing a chemical and/or biological process comprising: a first layer of material comprising fluid passages that define a microfluidic structure; and a second layer of material comprising fluid passages that define a larger-scale fluidic structure; the layers being bonded such that the fluid passages of the first layer communicate with those of the second layer to define a fluidic device.

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FLUIDIC DEVICES

This invention relates to fluidic devices, especially microfluidic devices, and methods for forming such devices.

Microfluidic devices are devices that involve fluid flow on a relatively small scale, typically but not exclusively in the size range below a few hundred microns in size.

Designing and manufacturing microfluidic devices is problematic. In design, it is difficult to predict fluid behaviour on such a small scale, especially when the fluids in question are suspensions (such as blood). As a result, in many circumstances a trial and error process is used to design microfluidic devices. This requires numerous devices to be manufactured and tested. However, because of the devices' small dimensions the manufacture of microfluidic devices is difficult, expensive and time-consuming. This in turn makes even the trial and error design stage troublesome. There is therefore a need for improved methods of manufacture of microfluidic devices that may not only assist in the production stage but also streamline designing so that prototypes of microfluidic devices can be made rapidly.

Microfluidic devices can be formed on silicon substrates. Conventional silicon fabrication techniques such as plasma etching and photo-masking are used to define small channels, holes or reservoirs in the surface of a silicon wafer. Then the channels can be sealed by bonding a sheet of material to the wafer's surface. However, silicon substrates, and the machinery needed for fabricating them in this way, are expensive; the shaping process takes a considerable time; and the substrates cannot be reused if the design is unsuccessful or a mistake is made in the manufacturing process. In addition, it takes a considerable time to set up the fabrication process, so it can take several weeks before the first wafer is shaped. This makes this route problematic for producing prototype microfluidic devices to commercial time-scales.

Microfluidic devices can be formed on substrates of plastics materials such as PMMA (Perspex). Known techniques for forming such devices are hot embossing and micro-injection moulding. The hot embossing technique involves first producing a pressing tool on which the desired microfluidic device structure is formed in negative relief, heating that tool and then pressing it into a plastics substrate to form the device in impression on the substrate. Alternatively the substrate itself may be heated until it becomes semi-fluid, for example PMMA may be heated to above its glass transition temperature (T_g), for instance to around 150°C. (T_g of PMMA is around 105°C). The pressing tool is usually formed from a silicon wafer so this technique generally suffers from many of the problems of the silicon processes described above, including in particular the long lead time before prototype devices can be formed. Micro-injection moulding requires a complex mould, which is generally difficult to produce, and is therefore not suitable for prototyping or short production runs. Other techniques for forming microfluidic devices on plastics substrates include laser-forming and pouring plastics material (such as silicone rubber) in liquid form over a mould structure, to whose shape the plastics material then sets.

There is thus a need for an improved technique for forming microfluidic devices, especially to allow rapid prototyping.

One example of a field in which microfluidic devices may be particularly useful is in microbiological analysis. Suitable microfluidic devices may assist in processes such as analysis of blood, lysing of cells and extraction of DNA. Until now the difficulties of designing microfluidic devices capable of reliably processing suspensions of cells have been very substantial.

According to one aspect of the present invention there is provided a method for forming a microfluidic device element, comprising impressing structures of a plurality of individual microfluidic devices on to a substrate.

The devices may be impressed by a hot pressing technique. Preferably the microfluidic devices are impressed by a die.

Preferably at least some of the plurality of devices have common structural microfluidic features.

The substrate is suitably in the form of a sheet. The substrate may suitably be of a plastics material such as PMMA. The substrate may suitably be of a thermoplastic material. The substrate may suitably me of a transparent or translucent material, or may be of an opaque material.

The device element may be a prototype device element, and the method may then be a method of forming a prototype device element.

Respective fluid input and output structures are preferably defined on the substrate for each individual microfluidic device. Preferably each microfluidic device is isolated from the other microfluidic devices within the dimensions of the substrate.

According to a second aspect of the present invention there is provided apparatus for performing a chemical and/or biological process, comprising: an analysis unit comprising a microfluidic device configured to perform at least one step in the process; and a fluid control unit in releasable fluid communication with the analysis unit and comprising flow control apparatus for causing fluid flow in the analysis unit so as to cause the process to be performed in the analysis unit; and isolation means for resisting the flow of fluid from the analysis unit to the fluid control unit.

The isolation means may comprise control means for controlling the flow control apparatus. The control means may comprise a data processing unit and a memory for storing a program for causing the processor to operate to control the

fluid control unit. The control means may be a personal computer. The control means may be loaded with software adapted to allow it to control the flow control apparatus. The analysis unit and/or the flow control unit preferably comprises sensors, which may be coupled to the control means. The processor may then be operable in response to signals received from the sensors. Fluid control may be by, but is not limited to, valve control and/or electrokinetics.

The isolation means preferably comprises at least one valve between the analysis unit and the fluid control unit. The isolation means may be for preventing the flow of fluid from the analysis unit to the fluid control unit.

The fluid control unit may comprise one or more pumps for causing fluid flow in the analysis unit. The fluid control unit may comprise one or more fluid reservoirs and/or connections for receiving removable fluid reservoirs such as bottles of reagents. Such a pump is suitably capable of causing fluid to flow from such a fluid reservoir to the analysis unit.

The analysis unit suitably comprises one or more containers for receiving waste material from the process. The analysis unit suitably comprises one or more recesses at its surface for receiving material to be processed. The analysis unit suitably comprises one or more recesses at its surface for holding a product of the process.

The analysis unit suitably comprises at least three sheets of material, each sheet comprising fluid passages, the sheets being joined together so that the fluid passages interconnect. One of the sheets suitably comprises a fine-scale microfluidic structure. The sheets may be all of the same material; alternatively, at least one of the sheets, suitably one that comprises a fine-scale microfluidic structure may be of another material. The fine-scale microfluidic structure may be formed by hot embossing or by silicon etching or by other methods. One of the sheets may comprise an intermediate-scale fluidic structure.

According to a third aspect of the present invention there is provided a microfluidic device comprising: a first layer of material comprising fluid passages that define a microfluidic structure; and a second layer of material comprising fluid passages that define a larger-scale fluidic structure; the sheets being joined such that the fluid passages of the first sheet communicate with those of the second sheet to define a fluidic device.

The microfluidic structure may be defined in a surface of the first layer of material, which is suitably a planar surface.

The microfluidic structure preferably comprises elements or features having dimensions smaller than 50μm or smaller than 10μm. The elements or features may include walls and/or gaps. The microfluidic structure suitably comprises cell trapping elements, which could be or comprise recesses or gaps of a width in the range from 2μm to 6μm.

Preferably none of the fluid passages of the second layer has a dimension of less than 50μm.

The microfluidic structure may be formed in the first layer of material by moulding, etching etc. into plastics material or a hard material such as silicon or another material. The said larger-scale fluidic structure may be formed in the second layer by a machining process such as drilling and/or milling, and preferably by using a mechanical cutting tool.

The device suitably includes at least one further layer of material comprising fluid passages that define a larger-scale fluidic structure.

The device may include a waste chamber in fluid communication with the microfluidic structure for receiving waste liquid, and preferably a vent passage communicating between the waste chamber and the environment. The vent

passage may have a barrier for resisting flow of liquid but permitting flow of gas through the vent passage.

The device may include an output chamber formed as a recess in one surface of the device, the output chamber being in fluid communication with the microfluidic structure via fluid passages in the device.

According to a further aspect of the invention there is provided a cell trapping structure comprising a first fluid passage and a second fluid passage and a plurality of channels extending between the first fluid passage and the second fluid passage; each channel having an inlet at its junction with the first fluid passage sized to permit cells to enter the channel, and having a variable cross-section for retaining cells in the channels. Further preferred features of such a structure are set out in the claims.

The present invention will now be described by way of example with reference to the accompanying drawings, in which:

figure 1 is a schematic view of apparatus for microfluidic analysis;
figure 2 is a cross-section of a fluidic unit of the apparatus of figure 1;
figure 3 is a plan view of a microfluidic layer of the unit of figure 2;
figure 4 is a plan view of a meso layer of the unit of figure 2;
figure 5 shows a microfluidic device;
figure 6 illustrates an electrode arrangement in the device of figure 6;
figure 7 is a partial cross-section of another fluidic unit;
figure 8 is a plan view of a switching layer of the unit of figure 7;
figure 9 is a partial cross-section of another fluidic unit;
figure 10 to 14 illustrate cell trapping structures;
figure 15 shows a schematic diagram of a fluid device; and
figures 16 to 18 show fluidic device structures.

The apparatus of figure 1 is intended for performing a fluid process for extraction of nucleic acids from blood. The apparatus comprises a central control unit 1, an

interface unit 2, a fluid control unit 3 and a fluidic unit 4. The central control unit 1 is a personal computer running software that allows it to receive and process data on the fluid process, and to transmit signals for controlling the fluid process. The control unit 1 is electrically linked at 5 to the interface unit 2 which performs the necessary signal conversions to interface between the central control unit 1 and the units 3 and 4 via respective electrical connections 6 and 7. The fluid control unit 3 contains electrically actuable flow control equipment such as pumps and valves (shown schematically at 8) for controlling fluid flow through the fluidic unit 4. These are linked to the fluidic unit by fluid connections 9 which could be silicone rubber tubes. The flow control equipment is controlled electrically by the central control unit 1 by means of electrical connections between the two. The fluid control unit also contains reservoirs of chemicals (shown generally at 10) to be used in the fluid process and which can be pumped to the fluid unit by the flow control equipment 8. The reservoirs 10 are bottles that can easily be replaced by an operator when they are empty or exchanged when the equipment is to be used to perform a different fluid process. The fluid control unit 3 also contains sensors (shown generally at 11) that provide signals to the control unit 1 via the electrical connection 6 to allow the central control unit 1 to monitor the operation of the fluid control unit 3. The sensors 11 could include fluid pressure and flow sensors for monitoring the flow of liquid to the fluidic unit 4 and sensors for monitoring the level of the chemical reservoirs. Optical sensors arranged across the fluid passages could be used to minimise disruption of the fluid flow.

The fluidic unit 4 incorporates interconnected fluidic components for permitting the apparatus to perform the fluid process. Some of those fluid components are illustrated schematically in figure 1. The fluidic unit includes an input reservoir 12 formed as a depression in the upper surface of the fluidic unit for receiving blood to be processed; an extraction chamber 13 in which the nucleic acid is extracted from blood cells; an output reservoir 14 formed as another depression in the upper surface of the fluidic unit for holding extracted nucleic acid for removal by a user; a series of intermediate devices 15 such as flow channels, mixing chambers and reaction chambers for allowing the blood, nucleic acid, waste products and

other media such as reactants, buffers or catalysts to flow around the fluidic unit and which connect the other fluidic components of the fluidic unit; and a waste container 16 for receiving waste. To allow air to escape from the system (preventing blockages) the waste container is vented to outside by a thin port 17. To prevent potentially harmful fluids from escaping, the port could be covered by a gas-permeable membrane. The fluid connections 9 connect to the intermediate devices 15.

The waste container may be treated with a biocide for increased safety. Such a biocide is preferably adhered to the walls of the waste container. It should be noted that the layered structure of the fluidic unit allows such treatment to be performed relatively easily.

The fluidic unit 4 could include sensors 18 that provide signals to the central control unit 1 via the electrical connection 7 to allow the control unit 1 to monitor the operation of the fluidic unit 4. The sensors 18 could include fluid pressure and flow sensors for monitoring the flow of liquids in the fluidic unit 4 and sensors for monitoring chemical properties of the liquids during the extraction process. Again, optical sensors could be used. However, in some situations – as will be described below – it may be intended for the fluidic unit to be a disposable add-on to the fluid control unit 3. In those cases it is preferable for the fluidic unit to include as few sensors as possible (preferably no sensors) so as to reduce the cost of the disposable unit and to reduce the time taken to connect it to the fluid control unit 3.

To use the apparatus a user places a sample of blood in the input reservoir 12 and then activates the extraction process using the control unit 1 – for example by causing a processor in the control unit to run a program stored in a memory in the control unit that is capable of controlling the other units to carry out the extraction process. By way of the interface unit 2 the control unit controls the flow control equipment 8 in the fluidic control unit 3 to draw the blood from the reservoir 12 into the extraction chamber 13, to cause the necessary chemicals from the

reservoirs 10 to flow around the fluidic unit so as to perform the nucleic acid extraction step, and to force the extracted nucleic acid into the output reservoir 14, from which it can be collected by the user. During the process the control unit monitors the signals it receives from the sensors in the fluidic control unit 3 and the fluidic unit 4 to and uses that information in determining how to control the fluid control unit.

The control process run by the control unit 1 is preferably such that no fluids (especially no bio-fluids) flow from the fluidic unit 3 to the fluidic control unit 4, and waste from the process is retained in the fluidic unit 4, suitably in the waste chamber 16. Thus, none of the units 1, 2 or 3 is contaminated by the blood used during the process. When the extraction process is complete and the DNA has been collected from the output reservoir 14 the fluidic unit 4 can be unplugged from the connections 7 and 9 and disposed of. To perform another extraction process a fresh fluidic unit can be plugged into the same connections and the extraction process repeated anew. The fluidic unit can suitably be provided in the form of a replaceable cartridge. For ease of use, the connectors 9 and 7 could be joined to provide a single connector fitting that mates to the replaceable cartridge. The connector fitting could also include a housing that holds the cartridge in place. The connector fitting could be mounted rigidly to the housing of the fluid control unit for convenience; so that the cartridge can be plugged into the fluid control unit, simultaneously making the fluid and electrical connections to the cartridge and fixing the cartridge in place for performance of the fluid process.

Since the none of the units 1, 2 or 3 is contaminated during the process, and a fresh fluidic unit is used each time, there is no risk of one blood/DNA sample being contaminated by a previous sample. This is especially useful in forensic analysis, where contamination must be avoided if results are to avoid criticism for lack of reliability. Also, there is no need for the operator to perform any cleaning. This saves time and makes the process suitable for use in situations where there are no cleaning facilities. The fact that the entire process is performed under the

control of the control unit 1 makes the apparatus suitable for use by relatively unskilled operators.

By suitable choice of the control software in the control unit 1, the chemicals available in the fluid control unit 3 and the set of fluidic devices available in the fluidic unit 4 the apparatus could be used for other processes. For example, different fluidic units 4 could be provided for analysing the blood of different animals, or the apparatus could be adapted to allow other reactions such as the polymerase chain reaction (PCR) or capillary electrophoresis to be performed.

There are numerous ways in which the fluidic unit 4 could be made, but figure 2 shows a schematic cross-sectional view of one possible embodiment. The fluidic unit 4 of figure 2 is formed from four sheets of PMMA (poly-methylmethacrylate) 20-23 which have been shaped by various techniques and then laminated together. Typical suitable thicknesses for the layers 20-23 are in the range from 200μm to 5mm, and preferably around 1mm. instead of PMMA other materials such as PS (polystyrene) could be used. It is preferred that the materials are thermoplastic (to allow them to be formed by hot embossing), bio-compatible (to avoid problems during use with bio-fluids) and transparent (to allow the process to be observed as it takes place inside the fluidic unit). The layers need not all be formed from the same material.

Three of the sheets (sheets 20, 21 and 22) are shaped by machining – specifically drilling and milling using computer-controlled CNC machinery with small tools such as 100μm routing bits and 300μm drill bits. These tools are used to create a network of passages, mixing chambers, waste chambers etc. which will link to form the desired network 15 when the fluidic unit is assembled. Vertical channels of the network are formed by drilling through a sheet. Horizontal channels are formed by routing in the top or bottom surface of the sheet or by drilling from the side of the sheet. The network is of as small a volume as possible, so as to reduce dilution of the blood/DNA/RNA, reduce the amount of blood/DNA that is needed and increase yield from the process. CNC machining is a relatively quick

process that can be performed automatically by relatively inexpensive machinery. Although it is generally too imprecise for forming very small scale microfluidic devices (because typical step resolutions of economical CNC machinery is greater than around 8μm) this does not matter here because in the device of figure 2 CNC machining is used only to form meso-structures.

The fourth sheet (sheet 23) is formed by a precise route, such as hot embossing. Figure 3 illustrates the stages in the hot embossing process for forming sheet 23. First (step a) a die 31 is formed on a silicon or metal block. The die has the negative structure of the shape that is desired to be formed in the sheet 23. The die and/or the plastics sheet are heated and then the die is pressed into the plastics sheet (step b). Typically the heat used is only just above the glass transition temperature of the plastics sheet, so a force of several tons is used for pressing the die. The die and the plastics sheet are allowed to cool so that the sheet hardens into the impression of the die, and then the die is withdrawn. Finally (step c) the unimpressed regions of the sheet are cut away at the location of the dotted lines 32 in figure 3b to leave a precisely formed sheet including a precisely formed small-scale microfluidic device 26 in the extraction chamber 13.

Holes are drilled into sheets 20, 21 and 22 to receive dowels 25 for locating the sheets precisely with respect to each other. Dowels or corresponding holes could be pressed into the sheet 23 by formations on die 31. Silicone rubber O-rings 24 are fitted to sheet 21 to allow a secure but releasable seal to be made with the fluid connectors 9 when the unit is in use. To unite the sheets together they are heated to close to their glass transition temperature, laid one on the other and allowed to fuse together under pressure. Alternatively an adhesive such as epoxy resin, or a solvent bonding method, could be used.

The laminated configuration of the unit has two major advantages. First, lamination allows complex three-dimensional networks of passages inside the unit to be built relatively easily. Second, lamination allows a highly efficient division of shaping processes. The extraction chamber, which contains small scale microfluidic devices, is formed in the lowermost sheet 23. There is no need for

any machining of that sheet. Therefore, the risk of the small scale devices being clogged or damaged by swarf from a machining process is small. The individual hot embossing and CNC machining steps are quick (once the hot embossing die has been made) and can easily be automated to allow the fluidic unit 4 to be produced economically. This is especially importance because the fluidic unit 4 is intended to be a disposable cartridge and should therefore be inexpensive to manufacture in volume.

Figure 4 shows a plan view of base layer 23, illustrating the extraction chamber 13 and (not illustrated to scale) its small-scale microfluidic device 26. The extraction chamber 13 is intended for receiving blood from the input reservoir 12, pinning the white blood cells whilst the red blood cells are washed into the waste chamber 16 by chemicals from the chemical reservoir 10 and whilst the white cells are lysed to release their nucleic acid, and then allowing the nucleic acid to be pumped into the output reservoir 14. The red cells are removed because the haemoglobin they contain would hinder a subsequent polymerise chain reaction. The extraction chamber comprises a generally linear central section that extends between two pairs of lobe-shaped chambers 30. The lobe-shaped chambers are intended to be located in fluid communication with through-bores 33 (see figure 2) in the overlying layer so that fluids can enter and leave the extraction chamber. The lobes are somewhat enlarged to allow some margin for error in the lateral locating of the sheets, and to allow fluids to be mixed, if necessary, in the lobes. The central section of the extraction chamber comprises two side passages 28, 29 that run between the pairs of ports. Between the side passages are walls 27 which are dimensioned and spaced so as to be able to trap white blood cells but not red blood cells.

Figure 5 shows the arrangement of the walls 27 in more detail, in a partial cut-away view. The spacing between the walls 27 is suitably in the range from 2μm to 6μm, and preferably around 4μm or 5μm. With a spacing in that range it is likely that when human blood is passed from one of the chambers 30 at one end of the central section to one of the chambers 30 at the other end of the central section

white cells will be trapped between the walls (as illustrated by dotted lines at 35 in figure 5). That spacing should also be sufficiently large to allow red blood cells to be washed through and not trapped. Human red blood cells are generally around 8μm in diameter and around 1.6μm in height and highly deformable, whereas human white blood cells are generally around 6μm in diameter and more rigid (although they are capable of limited changes in their morphology). If the walls 27 extend sufficiently high from the base 36 of the central section it may be possible to trap more than one white cell, one above the other, between the ends of each pair of walls (see figure 5).

Suitable dimensions of the extraction chamber for use with human blood are:

- thickness of walls 27: 5μm
- spacing between walls 27: 5μm
- width of channels 28, 29: 10μm
- depth of walls 27 and channels 28, 29: 10 to 80μm
- number of walls 27: roughly 3000 (dependant on the amount of DNA and therefore the number of trapped white cells needed)

Other dimensions may be preferred for similar microfluidic devices for trapping other types of cells, including non-human cells. The trapped cells are preferably nucleated cells.

When the layers of the fluidic unit 4 are assembled the flat surface of the overlying layer 22 seals the top of the extraction chamber. The walls 27 abut the overlying layer 22, supporting it and preventing it from slumping into the extraction chamber.

In use, the blood is forced repeatedly up and down the passages 28, 29 until enough white cells are believed to be trapped. Then the remaining blood, including the red cells, is washed away and the step of lysing the white cells can begin. This is done, for example, by the use of a lysing agent, which could be a surfactant such as sodium dodecylsulphate (SDS). As illustrated in figure 6 electrodes 40, suitably formed of platinum, may be fitted across the channel, and

connected via the interface unit 2 to the control unit 1, to allow the control unit 1 to apply an electrical field (e.g. with a high field gradient) that may assist in rupturing the trapped cells. The electrodes 40 may also be used earlier in the process to estimate the number of trapped cells (by measuring the resistance between the electrodes) and thereby determine when sufficient cells are trapped that the remaining blood can be washed away.

Figure 12 shows a schematic diagram of the structure of a further example of a fluidic unit for processing blood to obtain nucleic acid. The fluidic unit is indicated at 70, and can be provided by an integrated unit, preferably formed with a multilayer structure as described above. The valves 71-75 mounted in the fluidic unit are miniature but conventional valves. Externally of the fluidic unit are a well or reservoir B which holds reagent (this could if desired be integrated with the fluidic unit) and a waste container. Fluid control is provided by external electrical control of the valves and the application of pneumatic overpressure to inlets 76 and 77. Electrical control of the valves is performed under computer control in accordance with a predetermined sequence.

In operation, the fluidic unit is connected to the external units (e.g. the electrical connection to the valve controller and the pneumatic connection to a pressure source). A finger-prick sample of blood is added to well A. Then the entire fluid circuit is primed with reagent from well B by means of overpressure applied at 77 The chip is primed with blood cell suspension from well A by forcing the blood into the microfluidic cell filtering structure 78. The cell suspension is filtered in order to trap white blood cells via the application of a pressure differential across filter arrays within the unit. After a predetermined time, or when the filter is blocked, the system is purged of red blood cells via sequential flushing of the fluid circuit with reagent from well B. The white blood cells remain trapped in the specially designed channels of the microfluidic structure.

Three subsequent processes are available for lysing of the cells. In a first process the isolated white blood cells are lysed chemically by flushing lysis solution from

well C through the microfluidic structure. Then the cell lysate is flushed with reagent from well D to reservoir E within the substrate for post processing. In a second process the isolated white blood cells are lysed chemically by flushing lysis solution from well C through the microfluidic structure. Then nucleic acid is removed electrokinetically from the chip to area E within the substrate for post processing, via the application of one or more electrical potential differences between two or more appropriately placed electrodes on the substrate. In a third process the isolated white blood cells are lysed electrically by application of a potential difference across two appropriately located electrodes on the substrate. Then nucleic acid is removed electrokinetically from the chip to area E within the substrate for post processing, via the application of an electrical potential difference(s) between two or more appropriately placed electrodes on the substrate.

Further fluidic structures for postprocessing the nucleic acid may be integrated in the same fluidic unit as is used for separation of nucleic acid. Such postprocessing may include amplification and/or analysis, for example by means of microarrays. A valve, most preferably a microvalve, is preferably located between the separation section and the postprocessing section. The valve may be a one-way valve, to resist back flow from the postprocessing section to the separation section. Alternatively, the valve may be capable of resisting flow from the separation section to the postprocessing section until it is remotely actuated to allow such flow. The valve is preferable a miniature valve. Examples of valves that may be appropriate in specific circumstances include membrane valves, for example in which a flexible membrane includes a piercing which opens to allow flow through it only under relatively high pressure; valves in which a magnetically susceptible blocking member can be drawn out of a blocking position in a channel by means of an electromagnet set near the channel; and heat actuated valves, in which a blocking member can be melted by application of electrical current to a nearby heating coil.

Figures 16 to 18 show examples of multilayer hybrid microsystems having fluidic circuit containing mesofluidic elements 90 integrated with one or more microfluidic components 91. Tubing 92 provides fluid connectivity to the systems. The microfluidic components could be made, for example, of plastics material such as PMMA or of silicon. The mesofluidic components could be made, for example, of PMMA. The microfluidic components could be closed by a sheet 93 of, for example, glass.

The purpose of the mesofluidic elements is to provide fluid connectivity between the microfluidic components and small but conventional fluid components used for fluid control. Provision of fluid delivery networks and manifolding within the mesofluidics allows for lower consumption of material area during fabrication of any microfluidic components.

Cross-sectional dimensions within the mesofluidic components can be in the range of 10μm to 10mm, typically 200μm. Multilayer mesofluidics can be formed by the bonding of two or more layers of suitably processed material. Examples of such layers would be conventionally milled acrylic or moulded polydimethylsiloxane (PDMS). Joining of the layers can be achieved by direct bonding of surfaces following suitable surface preparation.

The microfluidic component comprises of a fluid system containing micron-dimensioned features. Cross-sectional dimensions of such features can generally range between 0.5μm and 500μm, typically being 3.5μm wide and 30μm deep. A specific use of features of such dimensions would be isolation of a particular cellular species from a mixed cellular sample for example the isolation of white blood cells from whole blood.

The microfluidic component can also contain fluid channels suited to delivering fluid to the micron-dimensioned features. Such channels would generally have cross-sectional dimensions in the range of 5μm to 10mm, typically being 200μm wide and 30μm deep.

The microfluidic component can be fabricated by etching of silicon, hot-embossing of polymer using an etched silicon tool or moulding of PDMS against an etched silicon tool.

In the case of silicon microfluidic components fluidic connectivity between the meso and microfluidic components can be achieved via direct bonding of the adjoining surfaces, or via the introduction of an intermediate layer of suitably processed material. Such layers could be diamond drilled or ultrasonically drilled glass anodic bonded to the silicon. This composite silicon/glass structure can be bonded to the mesofluidic component via direct bonding, by the application of a suitable adhesive to the surfaces or bonded by the introduction of a suitably processed adhesive layer between the surfaces.

In the case of acrylic or PDMS microfluidic components the meso and microfluidic components can be direct bonded following suitable surface preparation or bonded via the introduction of a suitably processed adhesive layer between the surfaces.

Fluid connectivity between the mesofluidic elements and conventional fluidic components such as valves can be achieved by gluing appropriately sized conventional tubing into drill-holes within the mesofluidic component.

As previously mentioned, it is very difficult to design microfluidic structures for suspensions such as blood because it is very difficult to model fluid behaviour as the structures' dimensions approach those of the suspended objects. Since the fluid behaviour cannot be modelled theoretically to sufficient accuracy it is normal design practice to produce prototype devices and test them to find whether they work properly. With the unit of figure 2 the process of designing microfluidic structures is much easier than before. With the unit of figure 2 a test base layer, whose surface contains many test microfluidic structures, can be made by hot embossing. Then only the upper layers 20, 21 and 22 need to be changed in

order to test all those structures and to optimise to each microfluidic structure the network defined in those upper layers. Since the upper layers are of lower precision than the base layer, they can be made quickly and with less specialised tools. This allows prototyping of microfluidic devices to be greatly accelerated, and for many test devices to be made at greatly reduced cost.

The hot embossed layer 23 need not be the same size as the other layers. In particular, it could be smaller than the other layers since the microfluidic device it contains might not occupy the whole of a full-size layer.

Another use for layers containing several microfluidic structures is to make the unit of figure 4 more flexible. The base layer of the unit (23 in figure 2) may be hot embossed to contain several small-scale microfluidic structures, each designed for use in a different specific process. With a suitably designed network in layers 20, 21 and 22 the control unit 1 could control which of the structures fluids are routed through, allowing the same disposable unit 4 to be used for different processes, under software control. Another approach is for a valve means to be provided to allow a user to mechanically switch between the structures. As an example of this approach, figure 7 shows a partial cross-section of an alternative fluidic unit to that of figure 2. In figure 7 the base layer is marked 50 and contains through-bores 51 that meet bores 33 to allow fluid to flow from bores 33 to the reverse surface of layer 50. On the reverse surface of layer 50 there are several small-scale extraction chambers 52, 53 containing small-scale microfluidic devices. Against that surface is a switching layer 54 which acts as the valve means. The upper surface of switching layer 54 contains routed passages 55-58. The switching layer is located relative to base layer 50 by dowels 59. When the switching layer is properly located the routed passages 55-58 link the bores 51 to the lobe-shaped input and output chambers of one of the chambers 52, 53. Before the extraction process takes place a user can move the switching layer to select a desired one of the chambers 52, 53 for use: for example, in figure 7 recess 59a is provided to receive a locating dowel when the switching layer is

moved to select chamber 52. Figure 8 shows a plan view of the switching layer 54.

The hot embossed layer could contain more than one copy of each design of microfluidic device, to allow for flexibility in designing the upper layers.

Other techniques than hot embossing could be used to form the layer that contains the fine-scale microfluidic device(s). Suitable techniques, such as micro-injection-moulding, should provide sufficiently high precision. Alternative techniques could be used to form the other layers. One possibility is to hot emboss or injection mould those layers too. It is also the case that by avoidance of the complicating requirement to form mesofluidic structures, simple silicon processing could be employed to form the fine-scale structures directly. Thus the main body of the device could be formed from a plastics or other material (e.g. by machining) and the layer(s) in which precision and/or fine-scale structures are to be formed could be of another material that is suitably advantageously suited to defining such structures. That material is preferably hard, with an even and fine-scale microstructure. It could be of a single crystal or a glass. Examples of suitable materials for a layer of that type including fine-scale structures include silicon. The layer could be formed by known precision forming methods such as etching, as is described above in relation to silicon wafers.

One way in which the microfluidic structures (e.g. of the multiple chambers 52, 53) could vary is to have cell entrapment structures (analogous to the gaps between walls 27 in figure 5) of different sizes for trapping cells of different sizes and different types or from different plants or animals. It might be preferred to provide more than one type of cell entrapment structure in a single microfluidic structure, to potentially increase the chance of trapping cells. Of course none of the chambers has to include any cell entrapment structures – the microfluidic structures could be for any appropriate purpose.

The extraction chambers and fine-scale microfluidic devices within them may take any suitable form. Some examples of alternative designs will now be described.

Figure 9 shows part of a unit that is analogous to that of figure 2. In figure 9 layers 60 and 61 are analogous to layers 22 and 23 respectively in figure 2. Layer 61 is formed with a fine-scale microfluidic device 62 by hot embossing. The device is similar to that of layer 23 but has no lobe-shaped input/output chambers. Instead, corresponding coupling structures 63 are formed by routing into the lower surface of layer 60. This structure may be preferred over that of figure 2 because the hot embossed structure (which is less flexibly fabricated) contains fewer features; therefore more flexibility in design can be provided by varying the easily-fabricated upper layers including layer 60.

The channels between the walls 27 in figure 5 are straight and arrayed in parallel with the blood cell suspension input of the chip and perpendicular to the red blood cell (RBC) removal flow stream in operation. The channels act as a simple filter and rely on trapped white blood cells (WBCs) 'sticking' to the channels, rather than exploiting cytomechanical effects to keep the cells trapped during RBC flushing. Figure 10 shows a plan view of such a structure. Typical dimensions are:

A: 1.5μm to 10μm, typically 3.5μm wide
B: Length: 2μm to 500μm long, typically 25μm long
Depth: 2μm to 500μm, typically 30μm

In another variation, the walls 27 in figure 5 could be made V-shaped in plan view rather than straight. This may aid entrapment of cells. Furthermore if the structure is made by hot embossing, such V-shaped structures will ease the release of the embossing tool from the substrate.

Figure 11 shows a scanning electron micrograph (SEM) of another channel design. These channels are profiled (as shown in the SEM) on the leading edge

and angled towards the direction of the red blood cell flushing flow such that any white blood cells trapped in the entrance of a channel will experience a net force which forces them further into the channel during red blood cell flushing. This can increase trapping efficiency and yield. Figure 12 shows a plan view of such a structure. Typical dimensions are:

A: 10μm to 500μm typically 25μm

B: 1.5μm to 10μm, typically 3.5μm

C: 1.5μm to 20μm, typically 10μm

D: 0μm to 500μm, typically 10μm

Depth: 2μm to 500μm, typically 30μm

Figure 13 shows a scanning electron micrograph (SEM) of another channel design. These channels have a variable cross-section which exploit the greater viscosity of the white blood cell constituents compared to red blood cells. The viscous properties of the cell determine the rate at which a cell can deform and relax to conform to its environment. The red blood cells pass through the length of the channels easily however once the leading edge of the white blood cells get past the short restriction at the channel entrance they expand into the wider section. They are prevented from moving forward during filtering or backwards out of the channel during red blood cell flushing because of their slow mechanical response to the restrictions at the ends of the channels. This can increase trapping efficiency and yield. The wider section is bounded by a wall 100 transverse to the channel on the inlet side, a wall 101 parallel with the channel, a wall 102 transverse to the channel on the outlet side and the rear face 103 of the partition defining the adjacent channel. The wall 102 is less transverse to the channel than the wall 102.

The channel arrays are also angled with the direction of the red blood cell flushing flow as above.

Figure 14 shows a plan view of such a structure. Typical dimensions are:

A: 5μm to 500μm, typically 35μm

B: 1.5μm to 10μm, typically 3.5μm

C: 2μm to 10.5μm, typically 5.5μm

D: 3μm to 498μm, typically 17μm

E: 1μm to 50μm, typically 6μm

F: 1μm to 50μm, typically 12μm

Depth: 2μm to 500μm, typically 30μm

A single fluidic unit may contain more than one of such structures.

The applicant draws attention to the fact that the present invention may include any feature or combination of features disclosed herein either implicitly or explicitly or any generalisation thereof, without limitation to the scope of any of the present claims. In view of the foregoing description it will be evident to a person skilled in the art that various modifications may be made within the scope of the invention.

CLAIMS

1. Apparatus for performing a chemical and/or biological process comprising:

a first layer of material comprising fluid passages that define a microfluidic structure; and

a second layer of material comprising fluid passages that define a larger-scale fluidic structure;

the layers being bonded such that the fluid passages of the first layer communicate with those of the second layer to define a fluidic device.

2. Apparatus as claimed in claim 1, wherein the microfluidic structure is defined in a surface of the first layer of material.

3. Apparatus as claimed in claim 2, wherein the said surface is a planar surface.

4. Apparatus as claimed in any preceding claim, wherein the microfluidic structure comprises elements having dimensions smaller than 50μm.

5. Apparatus as claimed in claim 4, wherein the microfluidic structure comprises elements having dimensions smaller than 10μm.

6. Apparatus as claimed in any preceding claim, wherein the microfluidic structure comprises cell trapping elements.

7. A microfluidic structure as clamed in claim 6, wherein the cell trapping elements comprise recesses of a width in the range from 2μm to 6μm.

8. A microfluidic structure as claimed in any preceding claim, wherein none of the fluid passages of the second layer has a dimension of less than 50μm.

9. A microfluidic structure as claimed in any preceding claim, wherein the microfluidic structure is formed in the first layer of material by moulding.

10. Apparatus as claimed in any preceding claim, wherein the larger-scale fluidic structure is formed in the second layer by a machining process.

11. Apparatus as claimed in claim 10, wherein the machining process is drilling.

12. Apparatus as claimed in any preceding claim, wherein the device includes at least one further layer of material comprising fluid passages that define a larger-scale fluidic structure.

13. Apparatus as claimed in any preceding claim, including a waste chamber in fluid communication with the microfluidic structure for receiving waste liquid.

14. Apparatus as claimed in claim 13, including a vent passage communicating between the waste chamber and the exterior of the device.

15. Apparatus as claimed in claim 14, wherein the vent passage has a barrier for resisting flow of liquid but permitting flow of gas through the vent passage.

16. Apparatus as claimed in any of claims 13 to 15, wherein the waste chamber is treated with a biocide.

17. Apparatus as claimed in any preceding claim, including an output chamber formed as a recess in one surface of the device, the output chamber being in fluid communication with the microfluidic structure via fluid passages in the device.

18. Apparatus as claimed in any preceding claim, comprising postprocessing means defined in at least one of the first layer, the second layer and a further layer bonded directly or indirectly to one of the first and second layers and in fluid communication with the microfluidic structure via fluid passages in the device.

19. Apparatus as claimed in claim 18, wherein the postprocessing means is capable of being controlled to perform amplification and/or analysis of products of a process for which the microfluidic structure is adapted.

20. Apparatus as claimed in claim 18 or 19, wherein the postprocessing means is defined in the second layer.

21. Apparatus as claimed in claim 20, wherein the second layer defines an elongate channel at one of its major surfaces for permitting fluid communication between the microfluidic structure and the postprocessing means.

22. Apparatus as claimed in claim 18 or 19, wherein the postprocessing means is defined in a further layer.

23. Apparatus as claimed in claim 22, wherein the second layer includes a through bore between its major surfaces for permitting fluid communication between the microfluidic structure and the postprocessing means.

24. Apparatus as claimed in any of claims 18 to 23, wherein the postprocessing means comprises a microarray.

25. Apparatus as claimed in any of claims 18 to 24, comprising a valve located in the fluid path between the microfluidic structure and the postprocessing means for controlling fluid flow between the microfluidic structure and the postprocessing means.

26. Apparatus as claimed in claim 25, wherein the valve is a remotely actuable valve.

27. Apparatus as claimed in claim 25 or 26, wherein the valve is an electrically actuable valve.

28. Apparatus as claimed in any of claims 25 to 27, wherein the valve is a pressure sensitive valve.

29. Apparatus as claimed in any of claims 25 to 28, wherein the valve is a one-way valve for blocking flow from the postprocessing means to the microfluidic structure.

30. Apparatus as claimed in any preceding claim, wherein the microfluidic device is adapted for trapping white blood cells.

31. Apparatus as claimed in any preceding claim, wherein the larger scale fluidic structure is a mesofluidic structure.

32. A method for performing a chemical and/or biological process comprising applying a biological sample to apparatus as claimed in any preceding claim, controlling the apparatus to cause at least part of the sample to pass through the fluid passages to the microfluidic structure, applying a reagent to the sample in the microfluidic structure, and controlling the apparatus to cause product to pass out of the microfluidic structure.

33. A method as claimed in claim 32, wherein the sample comprises blood cells.

34. Apparatus for performing a chemical and/or biological process, comprising:

an analysis unit comprising a microfluidic device configured to perform at least one step in the process; and

a fluid control unit in releasable fluid communication with the analysis unit and comprising flow control apparatus for causing fluid flow in the analysis unit so as to cause the process to be performed in the analysis unit; and

isolation means for resisting the flow of fluid from the analysis unit to the fluid control unit.

35. Apparatus as claimed in claim 34, wherein the isolation means comprises control means for controlling the fluid control apparatus, for example by one or both of valve control and electrokinetics.

36. Apparatus as claimed in claim 35, wherein the control means comprises a data processing unit and a memory for storing a program for causing the processor to operate to control the fluid control unit,

37. Apparatus as claimed in claim 36, wherein the analysis unit comprises sensors, the sensors are coupled to the control means and the processor is operable in response to signals received from the sensors.

38. Apparatus as claimed in claim 36 or 37, wherein the fluid control unit comprises sensors, the sensors are coupled to the control means and the processor is operable in response to signals received from the sensors.

39. Apparatus as claimed in any of claims 34 to 38, wherein the isolation means comprises at least one valve between the analysis unit and the fluid control unit.

40. Apparatus as claimed in any of claims 34 to 39, wherein the fluid control unit comprises a pump for causing fluid flow in the analysis unit.

41. Apparatus as claimed in any of claims 34 to 40, wherein the fluid control unit comprises a fluid reservoir.

42. Apparatus as claimed in claim 41 as dependant on claim 40, wherein the pump is capable of causing fluid to flow from the fluid reservoir to the analysis unit.

43. Apparatus as claimed in any of claims 34 to 42, wherein the analysis unit comprises a container for receiving waste material from the process.

44. Apparatus as claimed in claim 43, wherein the container for receiving waste material is treated with a biocide.

45. Apparatus as claimed in claim 44, comprising a passage connecting the container for receiving waste material to the exterior of the analysis unit for venting the container.

46. Apparatus as claimed in claim 45, wherein a filter permeable to gas but impermeable to liquid is located across the said passage.

47. Apparatus as claimed in any of claims 34 to 46, wherein the analysis unit comprises a recess at its surface for receiving material to be processed.

48. Apparatus as claimed in any of claims 34 to 47, wherein the analysis unit comprises a recess at its surface for holding a product of the process.

49. Apparatus as claimed in any of claims 34 to 48, wherein the analysis unit comprises at least three sheets of material, each sheet comprising fluid passages, the sheets being bonded together so that the fluid passages interconnect.

50. Apparatus as claimed in claim 49, wherein one of the sheets comprises a fine-scale microfluidic structure.

51. Apparatus as claimed in claim 50, wherein the fine-scale microfluidic structure is formed by hot embossing.

52. Apparatus as claimed in claim 50, wherein the fine-scale microfluidic structure is formed by silicon etching.

53. Apparatus as claimed in any of claims 49 to 52, wherein one of the sheets comprises an intermediate-scale fluidic structure.

54. A method for performing a chemical and/or biological process comprising applying a biological sample to apparatus as claimed in any of claims 34 to 53, controlling the apparatus to cause at least part of the sample to pass through the fluid passages to the microfluidic structure, applying a reagent to the sample in the microfluidic structure, and controlling the apparatus to cause product to pass out of the microfluidic structure.

55. A method as claimed in claim 54, wherein the sample comprises blood cells.

56. A method for forming a microfluidic device element, comprising impressing structures of a plurality of individual microfluidic devices on to a substrate.

57. A method as claimed in claim 56, wherein the devices are impressed by a hot pressing technique.

58. A method as claimed in claim 56 or 57, wherein at least some of the plurality of devices have common structural microfluidic features.

59. A method as claimed in any of claims 56 to 58, wherein the substrate is in the form of a sheet.

60. A method as claimed in any of claims 56 to 59, wherein the substrate is of a plastics material.

61. A method as claimed in any of claims 56 to 60, wherein the substrate is of a thermoplastic material.

62. A method as claimed in any of claims 56 to 61, wherein the microfluidic devices are impressed by a die.

63. A method as claimed in claim 62, wherein the device element is a prototype device element.

64. A method as claimed in any of claims 56 to 63, wherein respective fluid input and output structures are defined on the substrate for each individual microfluidic device.

65. A method as claimed in any of claims 56 to 64, wherein each microfluidic device is isolated from the other microfluidic devices within the dimensions of the substrate.

66. A cell trapping structure comprising a first fluid passage and a second fluid passage and a plurality of channels extending between the first fluid passage and the second fluid passage; each channel having an inlet at its junction with the first fluid passage sized to permit cells to enter the channel, and having a variable cross-section for retaining cells in the channels.

67. A cell trapping structure as claimed in claim 66, comprising an inlet for receiving a fluid for flushing cells through the first fluid passage in a flushing direction away from the inlet, and wherein the channels are angled to the first fluid passage away from the flushing direction.

68. A cell trapping structure as claimed in claim 66 or 67, wherein the minimum cross-sectional dimension of the inlet of each channel is in the range from 1.5μm to 20μm.

69. A cell trapping structure as claimed in claim 68, wherein the minimum cross-sectional dimension of the inlet of each channel is in the range from 2μm to 4μm.

70. A cell trapping structure as claimed in claim 68, wherein the channels narrow in the direction away from their inlets.

71. A cell trapping structure as claimed in claim 70, wherein the minimum cross-sectional dimension of the inlet of each channel is in the range from 8μm to 12μm,

and each channel has an outlet at its junction with the second fluid passage, and the minimum cross-sectional dimension of the outlet of each channel is in the range from 2μm to 8μm.

72. A cell trapping structure as claimed in any of claims 66 to 69, wherein each channel includes an outlet at its junction with the second fluid passage and the channels include a widened trapping cavity between the inlet and the outlet.

73. A cell trapping structure as claimed in claim 72, wherein the minimum cross-sectional dimension of the trapping cavity is greater than those of the inlet and the outlet.

74. A cell trapping structure as claimed in claim 73, wherein the minimum cross-sectional dimension of the trapping cavity is in the range from 2 μm to 10.5 μm.

75. A cell trapping structure as claimed in claim 73, wherein the minimum cross-sectional dimension of the trapping cavity is in the range from 4 μm to 7 μm.

76. A cell trapping structure as claimed in any of claims 66 to 75, wherein the cells are nucleated cells.

77. A cell trapping structure as claimed in claim 76, wherein the cells are white blood cells.

78. A method for trapping cells comprising flushing cells through a cell trapping structure as claimed in any of claims 66 to 77.

79. Apparatus for performing a chemical and/or biological process comprising:

a fluid inlet;

fluid passages that define a microfluidic structure in fluid communication with the inlet; and

a waste chamber in fluid communication with the microfluidic structure for retaining waste liquid.

80. Apparatus as claimed in claim 79, including a vent passage communicating between the waste chamber and the exterior of the device.

81. Apparatus as claimed in claim 80, wherein the vent passage has a barrier for resisting flow of liquid but permitting flow of gas through the vent passage.

82. Apparatus as claimed in any of claims 79 to 81, wherein the waste chamber is treated with a biocide.

83. A method for performing a chemical and/or biological process comprising applying a biological sample to apparatus as claimed in any of claims 79 to 82, controlling the apparatus to cause at least part of the sample to pass to the microfluidic structure, applying a reagent to the sample in the microfluidic structure, and controlling the apparatus to cause product from the action of the reagent to pass out of the microfluidic structure and to cause waste from the action of the reagent to pass to the waste chamber.

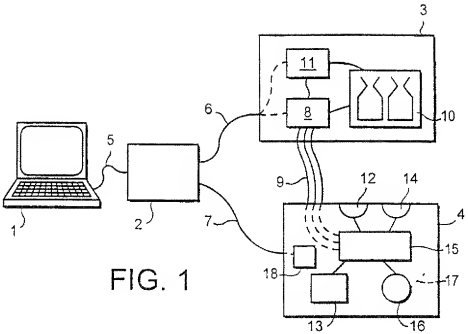
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FIG. 1

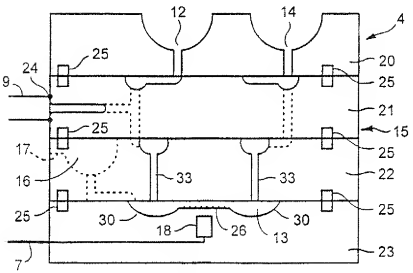
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FIG. 2

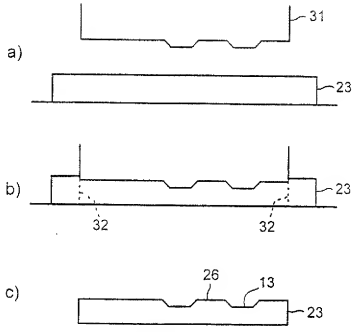


FIG. 3

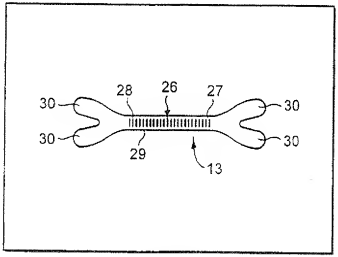


FIG. 4

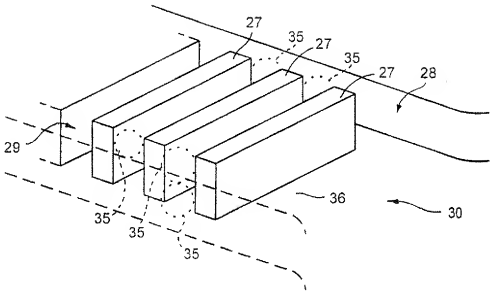


FIG. 5

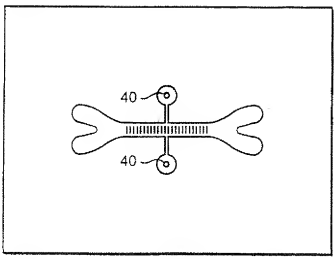


FIG. 6

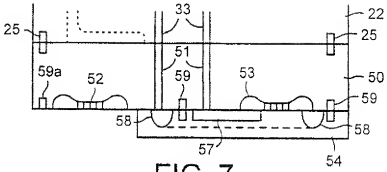


FIG. 7

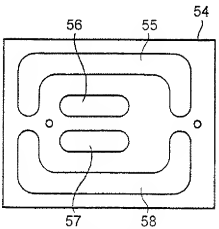


FIG. 8

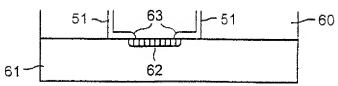


FIG. 9

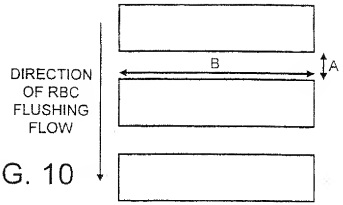


FIG. 10

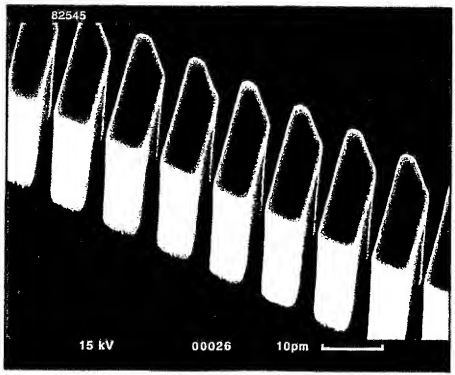


FIG. 11

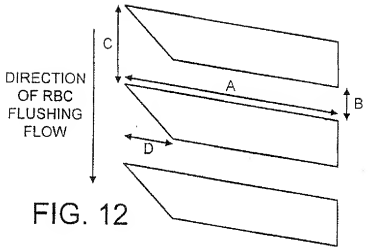


FIG. 12

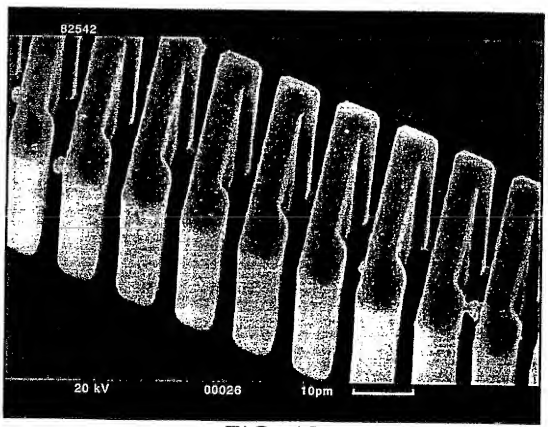
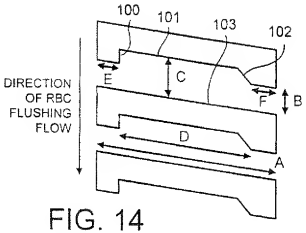


FIG. 13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DIRECTION
OF RBC
FLUSHING
FLOW
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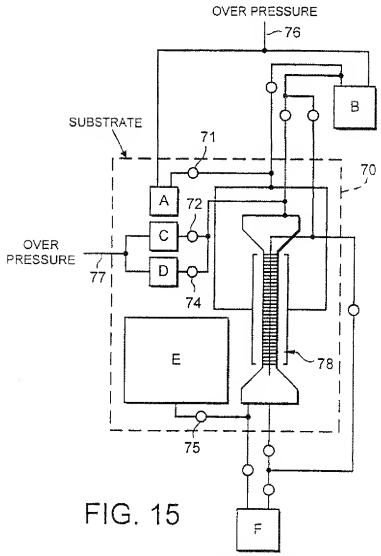
FIG. 14


OVER PRESSURE
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SUBSTRATE
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OVER
PRESSURE
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FIG. 15

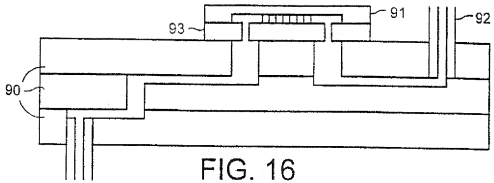

FIG. 16

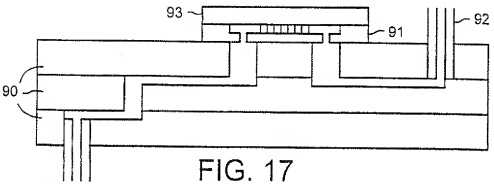

FIG. 17

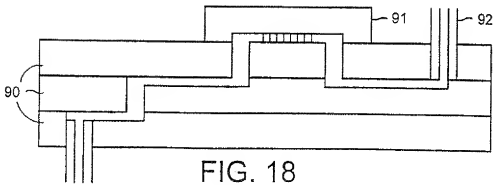

FIG. 18